embryos and embryogenic tissue out of the megagametophyte. If culture conditions are suitable, initiation proceeds and the extruded embryo or embryos undergo the process of cleavage polyembryony. This results in the formation of early stage somatic embryos in a glossy, mucilaginous mass.

[014] After embryogenic cultures are initiated, the somatic embryos are transferred to a second medium with an appropriate composition of plant hormones and other factors to induce the somatic embryos to multiply. In the multiplication stage, cultures can double up to 2-6 times in one week. Once large numbers of embryos are obtained in the multiplication stage, the embryos are moved to a development and maturation medium. Here, the correct balance of plant hormones and other factors will induce the early-stage embryos to mature into late stage embryos. Following the maturation and development stage, embryos are germinated to form small seedlings. These seedlings are then acclimated for survival outside of the culture vessel. After acclimation, the seedlings are ready for planting.

[015] The relative ability to propagate plants by somatic embryogenesis can vary greatly between species. Among conifers, for example, spruce (*Picea*) species and Douglas fir are easily propagated, while *Pinus* species are much more difficult. Many *Pinus* species, including Loblolly pine (*Pinus taeda*), do not readily initiate embryonic cultures. Typical initiation frequencies between 1% and 12% are reported for various *Pinus* species (Becwar et al., *For. Sci.* p1-18 (1988), Jain et al., *Plant Sci.* 65:233-241 (1989), Becwar et al., *Can. J. For. Res.* 20:810 (1990), Li and Huang, *J. Tissue Cult. Assoc.* 32:129 (1996)). Laine and David, (*Plant Sci.* 69:215 (1990)), however, were able to obtain high frequencies of initiation (up to 59%) in *Pinus*

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caribaea, suggesting that not all *Pinus* species are recalcitrant. Also, one earlier report described initiation frequencies of 54% in White pine (*Pinus strobus*). Finer et al., *Plant Cell Rep.* 8:203 (1989). However, other workers were not able to duplicate this success. Michler et al., *Plant Sci.* 77:111 (1991). The results in the literature demonstrate the recalcitrance of *Pinus* species, especially Loblolly pine, in regeneration by somatic embryogenesis.

[016] Nevertheless, once this process is understood from the standpoint of developmental genetics, breeders will then have the appropriate tools to monitor, intervene, and improve both the regeneration frequency and the overall quality of tree stock through genetic engineering. For example, both environmental requirements and responsiveness of a developing embryo change as the embryo passes various developmental milestones. Consequently, accurate and timely knowledge of the developmental stage of an embryonic culture would allow the skilled practitioner to beneficially adjust the growth media components and other environmental factors to achieve optimal embryo survival, growth, and maturation. In addition, an understanding of developmentally regulated genes would allow for early selection of advantageous clones and provide tools for developmentally regulated transgenic expression systems.

[017] Currently, a reasonable determination of the precise developmental stage of an embryo requires a practiced, physical familiarity with the morphological appearance of embryos at different stages, which is further complicated by the presence of morphological variations between species. Consequently, visual determination is performed best by experts in the field. Thus, there is a need in the art for a staging method which can be reliably practiced by the ordinary practitioner. The

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current invention will allow one to stage embryos based on a relational database system profiling gene expression patterns instead of physical morphological differences, thereby permitting one less skilled in the art of visual staging to biologically determine the stages of embryogenesis.

[018] The traditional morphological staging method provides only a crude indication of the underlying biochemical condition or state of an embryo. This level of information is insufficient for refining culture conditions, including media formulations, or for selecting potentially advantageous embryo clones for further development. Thus, there is a need in the art for a more sensitive staging method that precisely defines the physiological age, health, growth requirements, and potential fitness of a particular embryo. The current invention will allow definitive staging significantly beyond that currently practiced in the art, and provides a detailed analysis of the biochemical state and potential fitness of an embryo by comparison to developed relational database profiles.

[019] Visual staging methods depend on morphological markers to assign a numerical stage of 1-9 to an embryo. Nevertheless, it is well accepted that visually undetectable developmental changes occur in an embryo after it reaches stage 9. The current invention is particularly useful in providing means for monitoring and evaluating the developmental state of these older embryos, as genetic responses occur and are detectable up to and through an adult tree's life.

[020] There further exists in the art a need for information regarding the proteins, genes, and gene expression patterns in plant embryo development, as well as a more thorough understanding of how this information relates to the physiology,

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